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Eradication of Banana Viruses from Naturally Infected Banana Plants 2. Production of Certified Banana Plants and Virus Tested

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Abstract: For production certified banana plants and virus tested from naturally infected plants, RT-PCR was done to detect BMV and BBTV in shoot tips of banana cv. Williams. Meristem tip (apical dome) culture has been frequently used to eliminate BMV and BBTV. It was found that the survival plants were 20 and 15% while the virus free plants were 70 and 75% respectively. Two kind of therapeutic *in vitro* were applied. The micropropagated shoots were exposed to 36, 38 and 40°C for 30 days. The best temperature that gave virus-free plants was 36°C (100% for BBTV and 60% for BMV). On the other hand dry heat treatment of 40°C at one week is useful in curing the infected rhizomes effectively. Two antiviral compounds, ribavirin and salicylic acid at concentration 10 mg/L medium enhanced growth differentiation of the propagative meristem, while decreasing of virus free plants percentage. On the other hand 30 mg/L of two antiviral decrease the development of shoot micropropagated plantlets and had a deleterious effect on the regeneration and growth of the propagated shoots but increase of virus free plantlets, 90 with ribavirin and 93 and 90 with salicylic acid for BBTV and BMV respectively. Randomly amplified polymorphic DNA (RAPD) fingerprints was used to analyze somaclonal variations in tissue culture-derived banana plants. RAPD analysis showed genetic variations of the analyzed plants (10 regenerates). The genetic variations in approximately 4% were detected in 6 subculture and mother plants.

Key words: Banana plant, RT-PCR, RAPD-PCR, Tissue culture Therapeutic.

INTRODUCTION

The stage and condition of suckers prior to initiation may play a major role in further multiplication potential. The requirement for disease freedom is essential to avoid later quarantine problems when plants are moved from laboratory to the field.

In vitro the tissue culture is the method used to produce disease-free plants for two main reasons, firstly, this system produces plants directly from each clean sucker that has been both visibly inspected DPI staff to ensure it is free from symptoms of disease and laboratory tested to be sure it is free from virus. Secondly, the process of tissue culture requires the plant tissue to be sterilized. Tissue culture is a means of mass producing plants in a sterile, controlled environment. Every step in the process of multiplication in TC is carried out under sterile conditions. This ensures that the plants produced are clean but the process does not confer resistance to pest and disease[15]. Israeli *et al.* and Hamiz and Smith[17,13].

Techniques of modern molecular biology and tissue culture were used and grow for more than decade mostly for elimination of virus disease in planting material.

The analysis of tissue culture derived plants for somaclonal variations was yet to be established. Recently RAPD 6 molecular marker linked to somaclonal has been introduced in banana variations programmes.

Therefore, this study was conducted to employ tissue culture technique for production and micropropagation banana plants virus free and RAPD analysis as simple molecular marker tools for the analysis of somaclonal variations in tissue culture derived banana.

MATRIALS AND METHODS

The naturally infected banana plants (*Musa* spp. cv. Williams confirmed by PCR technique used as sources of meristems for tissue culture. These experiments were carried out in the tissue culture Lab., Virus and Mycoplasma Department; Plant Disease Institute, Agric. Research Center (ARC). BBTV and BMV infected banana plants grown under greenhouse conditions were used as a source for virus elimination.

Culture media: Media used in this investigation were salt mix of Murashige and Skoog's medium (1962) 4.4 g/L constituent required in large quantities, e.g., sucrose 30 g/L and agar 7 g/L were weight at the time of medium preparation. Hormones required depend on the stage carried on as Table (1).

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Table 1: Medium composition.

Constituents	Medium				
	Basal	Starting	Shooting	Pre-rooting	Rooting
MS salt g/l	4.2	4.2	4.2	4.2	4.2
Sucrose g/l	20	30	30	30	30
BAP mg/l	--	3	5	--	--
NAA mg/l	--	--	--	--	2
IBA mg/l	--	--	--	1	--
Myo-inositol g/l	0.1	0.1	0.1	0.1	0.1
pH	5.8-6.0	5.8-6.0	5.8-6.0	5.8-6.0	5.8-6.0

Table 2: Olifonucleotide primers for BBTV and BMV.

Virus	Nucleotide sequence
BBTV	Reverse 5`- GCTAGGTATCCGAAGAAATC-3` Forward 5`- TCAAACATGATATFTAATTC-3`
BMV	Reverse 5` - CCCCGGATCCTGGTCTCCTT-3` Forward 5` - CCCCGGATCCACATCAAGTTTTAG TTCAATTC-3`

Chemicals were dissolved into a liter of distilled water and the pH was adjusted to 5.8. Agar at the rate of 7 g/l was added to medium and then dispensed into Pyrex glass tuber containing 10 ml^3 (starting) and culture jars containing 40 ml^3 (Shooting and Rooting), then autoclaved at 121°C under pressure of 1.2 lb/inch/cm^2 for 20 min. All containers were kept for three days at room temperature before culture. All cultures in these investigations were incubated at 28±1°C under photo period cycle of 16/8 hr. as light/dark. Light intensity was used at 25000 Lux, with white fluorescent tubes.

Production of virus-free banana plants: The inoculated banana plants cv. Williams confirmed by PCR technique used as sources of meristems for tissue culture.

Meristem tip: Meristems of infected banana plant cv. Williams were excised from shoot apices with rhizomatous base (about 2.5 cm^2 X 5 cm length), washed under running tap water for 15 minutes. Meristems were surface sterilized by soaking in Clorox (commercial solution of sodium hypochlorite 5.25% active ingredient) at 15% for 30 minutes with shaking in shaker then rinsed several times in sterilized distilled water, containing citric and ascorbic acids (100 mg/l).

Individual meristems (the dome with 2 to 4 leaf primordia with rhizomatous base) were excised. The cut meristems were soaked in ethanol 70% for 5 seconds before transferred to the culture medium. Individual meristem (1 cm square X 1 cm length) was cultured in glass tubes containing starting medium (Table 1). The cultured tubes were incubated in a growth room under incubation conditions. The culture meristems were observed weekly. Monthly sub-cultured of the meristems to a fresh medium was carried on. Meristem-derived plantlets were tested for the presence of BBTV or BMV using DAS-ELISA technique. Virus-free plantlet percentage was recorded.

Chemotherapy: Meristems tip (about 3 mm long) were excised and cultured in basal medium (Table 1), the culture was supplemented with 10, 20, 30 and 40 mg/l of virazole, other group was supplemented with 10, 20, 30 and 40 mg/l of salicylic acid then the cultures were incubated as mentioned before. The growth index was calculated every week.

Heat therapy: *in vivo* Rhizomes 20-30 cm in diameter of viral infected banana plants cv. Williams and healthy plants were placed in polyethylene bags, added to peat and covered approximately two-thirds with peatmoss of the rhizomes then incubated at 40°C, the lateral buds which developed after 7, 10 and 15 days of heat treatment were removed and cultured as mentioned before.

In vitro meristems of about 5-mm length were cultured on shooting medium (sub-culture 1) medium. The resulted plantlets were exposed to 36, 38 and 40°C up to 10 days. The growth index and % of virus-free plantlets were recorded.

ELISA test: The virus isolates BMV and BBTV were detected in naturally infected plants and plantlets were resulted from tissue culture by DAS-ELISA according to Clark and Adams[7]. ELISA kits for detecting virus isolates were provided by Sanofi Sante Animal Paris, France.

Isolation of T-RNA of BMV infected banana leaves: RNA was extracted according to Gibbs and Mackenzie[12].

RT-PCR amplification: cDNA synthesis (reverse transcription from RNA of BMV) was synthesized using the CMV CP_5 as in Table (2). Complementary of the conserved ultimate 3` terminal 10 nucleotides of all CMV-RNA3 and Avian Myeloblastosis virus reverse transcriptase (AMV-RS). The PCR reactions were performed according to conditions and cycling parameters described by Quemada *et al*.[27].

Isolation of t-DNA of BBTV infected banana leaves was extracted using a version of CTAB method of Dellaporta *et al*.[8].

PCR amplification of DNA-BBTV: Ougonucleotide primers for PCR were derived from the published sequences of BBTV-DNA-4. The nucleotide sequences of two primers used in the PCR amplification were shown in Table (2). The PCR reactions were performed according to conditions and cycling parameters described by Harding *et al.*[14].

Isolation of genomic DNA and RAPD analysis: DNA was isolated from banana plants (mother plant) and plantlet (subculture 6) using the CTAB method of Doyle and Doyle[9]. PCR amplification was performed in 0.01

Table 3: Olifonucleotide sequences of the primers used.

Primer	Primer sequence 5 ----------3	Primer	Primer sequence 5 ----------3
OPA-02	TGCCGAGCTG	OPB-02	TGATCCCTGG
OPA-09	GGGTAACGCC	OPB-03	GATCCCCCTG
OPA-10	GTGATCGCAG	OPB-04	GGACTGGAGT
OPA-18	ACGTGACCGT	OPB-06	TGCTCTGCCC
OPC-05	GATGATCGCC	OPB-07	GGTGACGCAG
OPC-19	GTTGCCAGCC	OPD-05	TGAGCGGACA

cm³ reaction mixture containing 20 mg template DNA, 0.5 unit tag polymerase (Promega, USA), 200 µl each of dATP, dCTP, dGTP, dTTP, 10 pmole random primer (Table 3) and appropriate amplification buffer. The mixture was assembled on ice, overlaid with a drop of mineral oil. Amplification was performed for 45 cycles, using UNO thermal cycler of Biometra (Germany) as following: one cycle at 92°C for 3 min and then 45 cycles at 92°C for 30s, 35°C for 60s and 72°C for 2 min (for denaturation, annealing and extension, respectively). Reaction was finally incubated at 72°C for 10 min and further 10 min at 62°C.

The PCR products were analyzed by electrophoresis in 2% agarose in TAE buffer, stained with 0.2 µg cm³ ethidium bromide and photographed under UV light.

RESULTS AND DISCUSSIONS

Production of virus-tested: PCR protocol was done to detect BBTV and BMV in leaves of banana plants, which are used to produce virus-free plants and micropropagated shoots. Detect BBTV and BMV in infected banana plants using PCR protocol, Fig. (1) shows agarose gel electrophoresis analysis of PCR amplified products of BBTV and BMV with specific primer pairs. The size of BBTV-DNA amplified products was 500 bp (Fig. 1-A). The size of BMV-DNA amplified products was 600 bp max (Fig. 1-B). Data showed that BBTV and BMV were detected in 10 plants.

For establishment stage apical meristem (explants) were cultivated after sterilization process on media containing 0.1 mg/l BAP that mainly resulted in the development of meristem (Fig. 2-A). While the micropropagation stage were done by subculturing for plantlet to six times every 21 days (Fig. 2B). Before rooting, microshoots were cultivated on medium with 0.45 mg/l BAP that mainly resulted in the growth of pseudostem and leaves (Fig. 2-C). To induce rooting, shoots were wounded by removing a slice of epidermis (about 5 mm) of their base. Root formation was induced on rooting medium contains naphthalene acetic acid (0.4 mg/l). Roots initiated in a week and reached a level of 70-80% after 2-3 weeks.

Elimination of banana viruses: The infected plantlets developed from explants (meristem) were exposed to therapeutic as follows:

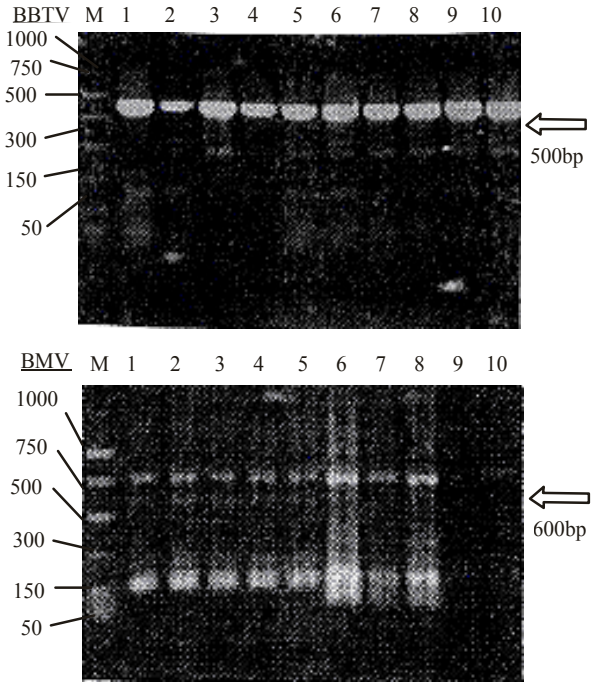

Fig. 1: Agarose gel (1%) electrophoresis analysis of PCR stained by ethidium bromide. BBTV: Amplification total DNA from infected plant materials (banana plants) using C -H and CP-C degenerate prime for BBTV-CP. The arrow indicates a 500 bp for the PCR products. Lane M PCR marker, Lane 1,2,3,4,5,6,7,8,9, 10 banana plants inoculated with BBTV isolate. BMV: Amplification total RNA from infected plant materials (banana plants using CP-H and CP-C specific degenerate primer for BMV- CP. The arrow indicates a 600 bp for the RT-PCR products. Lane M PCR marker, Lane 1,2,3,4,5,6,7,8,9,10 banana plants inoculated with BMV isolate.

Meristem tip culture has been frequently used to eliminate BBTV and BMV from infected plants dependence meristem size. The results in Table (4) showed that, the percentage of the survival plantlets were 15 and 20 as well virus-free plantlets BBTV and BMV were 75 and 70% respectively whereas tested with DAS-ELISA. While the survival of healthy plantlets ranged from 75-90% due to apical meristem.

Virazole and salicylic acid antiviral were incorporated individually into MS medium on which the micropropagated shoot tip of subculture 2.

Results demonstrated that virazole and salicylic acid at concentration 10 mg/l enhanced growth differentiation of propagated meristem. But decreasing of percentage virus-free plants. On the other hand, 30 mg/l of two

Table 4: Evaluation of therapeutic operation on virus-free banana plantlets via tissue culture.

	BBTV			BMV		
Treatment	No. of plantlets	% survival	% virus elimination	No. of plantlets	% survival	% virus elimination
Meristem tip culture	50	15	75	50	20	70
Chemotherap: Virazole						
10 ppm	50	70	70	50	80	65
20 ppm	50	65	75	50	75	71
30 ppm	50	62	90	50	70	90
40 ppm	50	62	90	50	71	90
Salicylic acid						
10 ppm	50	45	75	50	75	75
20 ppm	50	40	85	50	73	89
30 ppm	50	35	93	50	72	99
40 ppm	50	32	92	50	70	99
Thermotherapy: In vitro						
36°C	50	50	100	50	65	60
38°C	50	50	66	50	62	60
40°C	50	50	68	50	60	60
In vivo						
7 days	50	50	75	50	50	65
10 days	50	50	85	50	50	60
15 days	50	95	100	50	50	75

Tested with DAS-ELISA.

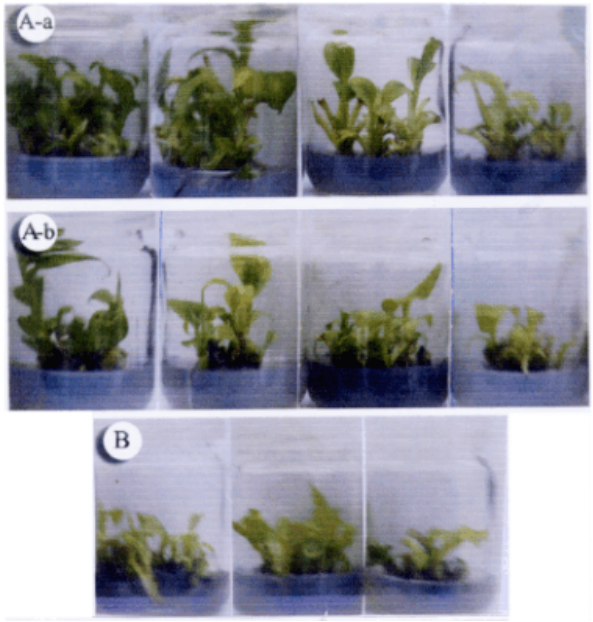

Fig. 2: Banana plantlets (subculture 2) infected with BBTV exposed to theraputic operations.
(Aa) Virasole at 10,20,30 and 40 ppm.
(Ab) Acetyl acetic acid at 10,20,30 and 40 ppm.
(B) Thermotherapy at 36,38 and 40°C.

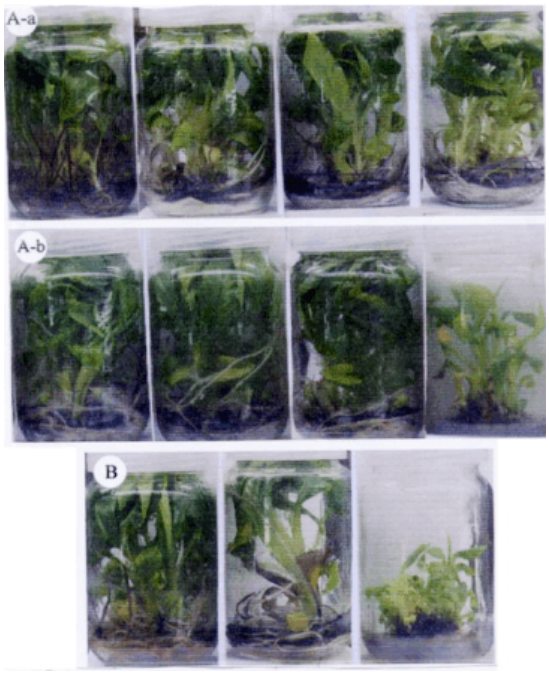

Fig. 3: The banana plantlets (subculture 3) regenerated from subculture 2.
(Aa) Virasole at 10,20,30 and 40 ppm.
(Ab) Acetyl acetic acid at 10, 20, 30 and ppm.
(B) Thermotherapy at 36,38 and 40°C.

antiviral decrease the development of shoot micropropagated plantlets and had a deleterious effect on the regeneration and growth of the propagated shoots (Figs 3 & 4 and Table 4) and increase of virus-free plantlets 93 and 99% whereas tested with DAS-ELISA for virazole and salicylic acid respectively. The phytotoxin effects of these compounds were particularly strong i.e. chlorophyll breakdown and inhibition of regeneration.

Thermotherapy: The heat treatment 40°C of infected rhizomas, *in vivo* the later buds which developed after 7 days of heat treatment were gave 100 and 75% of virus elimination BBTV and BMV respectively and conformed by +ve DAS-ELISA against IgG specific BBTV and BMV Another (samples). The micropropagated shoot tip of subculture 2 were exposed to 36, 38 and 40°C for 30 days under photo period cycle of 16/8 hours as light/dark. Then, the plantlets (subculture 3) were transferred to fresh MS medium and incubated at 28±1°C. The best temperature that gave virus-free plantlets (tested with DAS-ELISA) was 36°C (100% for BBTV & 60% for BMV (Table 4).

Certified plantlets: Random Amplified Polymorphic DNA (RAPD) analysis involves the amplification of small sequences of target DNA using random primer. The strength of RAPD analysis is able to assess genetic variation of characters for evaluation. This makes RAPD analysis as powerful tool for assessing genetic variation

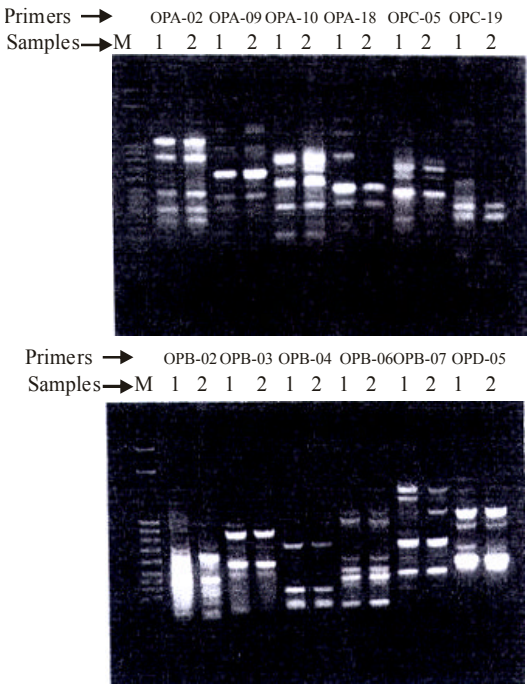

Fig. 4: Agrose gel (1%) electrophoresis analysis of RAPD-PCR amplified products from DNA shoot tips (subculture "1" & subcuture "6") of micropropagation banana using 12 random primers. M is standard DNA marker.

Table 5: Comparison of the genotype polymorphic (fingerprinting) between shoot (subculture "1" and subculture "6") of micropropagation banana plants *in vitro* with number molecular size type bp and type of the amplified DNA band generated by 12 random primers.

	Subculture 1*				Subculture 6*			
Primer sequence	TAF	CAF	SAF	% of poly-morphic	TAF	CAF	SAF	% of poly-morphic
OPA-02 TGCCGAGCTG	12	10	-	-	12	10	2—2000, 1800	16.5
OPA-09 GGGTAACGCC	5	5	-	-	5	5	-	-
OPA-10 GTGATCGCAG	8	7	-	-	8	7	1—*1450	-
OPA-18 AGGTGACCGT	6	3	3—1400, 800, 250	50	6	3	-	-
OPC-05 GATGATCGCC	8	6	2—*1400, 900	25	8	6	-	-
OPC-19 GTTGCCAGCC	8	6	2—*1700, 900	25	8	6	-	-
OPB-02 TGATCCCTGG	10	7	-	-	10	7	3—*750, 400, 350	30
OPB-03 GATCCCCCTG	10	7	2—*300, 250	20	10	7	1—*400	10
OPB-04 GGACTGGAGT	7	7	-	-	7	7	-	-
OPB-06 TGCTCTGCCC	11	11	-	-	11	11	-	-
OPB-07 GTGACGCAG	10	8	2—*1700, 800	20	10	8	-	-
OPD-05 TGAGCGGACA	9	9	-	-	9	9	-	-
Total	104	86	11	10.5	104	86	7	6.7

between the mother plant and subculture 6 of banana plantlets during banana propagation through tissue culture.

All primers used in the present study resulted in the appearance of PCR products with varied band numbers as shown (Fig. 4 and Tables 5- 12). A total amplified fragments (TAF) 104 DNA bands were detected across 12 random primers, 8 of them were polymorphic about 18% (Table 5). Primer OPA-02 yield 12 DNA bands with 16.5% polymorphism across the studied genotypes, the fragment sizes ranged from 6530 to 10330 bp. Primer OPA-09 showed 5 bands without polymorphic bands, the fragment size was 3900 bp. Primer OPA-10 produced 8 bands with 12.5% polymorphism, the fragment sizes ranged from 4350 to 580 bp. Primer OPA-18 showed 6 bands with polymorphic bands about 50%, the fragment sizes ranged from 2750 to 5200 bp. Primer OPC-05 revealed 8 bands with polymorphism about 25%, the fragment sizes ranged from 3000 to 5300 bp. Primer OPC-19, showed 8 TAF with 25% PAF, the fragment sizes ranged from 2300 to 4500 bp. Primer OPB-02 showed 10 TAF with about 30% PAF, the fragment sizes ranged from 3650 to 5150 pb. Primer OPB-03 yield 10 TAF with 30% PAF, the fragment sizes ranged from 5100 to 5250 bp. Primer OPB-04 produced 7 TAF without PAF, the fragment size was 3500 bp. Primer OPB-06 produced 11 TAF without PAF, the fragment size was 7300 bp. Primer OPB-07 yield 10 TAF with 20% PAF, the fragment sizes ranged from 7120 to 9620 bp. Primer OPD-05 produced 9 TAF without PAF, the fragment size was 805 bp.

The genotype specific markers for two subcultures 1 and 6 generated based on RAPD-PCR analysis are shown (Table, 5). As high as 11 and 7 (RAPD-PCR markers) out of 104 bands about 10 and 6.7% respectively were found to be useful as genotype specific markers, which present in one of subculture and absent in another subculture. The number markers of RAPD-PCR genotype specific markers (SAF or PAF) was scored 3 markers for primers OP-A18, OPB-02 and OPB-03, two markers for primers OPA-02, OPC-09, OPC-19 and OPB-07, one marker for OPA-10 and unexpected results were recorded by primers OPA-09, OPB-04, OPB-06 and OPD-05 while revealed no any genotype specific markers (SAF, Table 5). The

genotype markers (TAF) for two subcultures 1 and 6 generated based on RAPD-PCR analysis as shown (Table, 5) was 104 DNA bands, 86 DNA bands genotype monomorphic (CAF) out of 104 and 18 DNA bands genotype polymorphic (SAF). The fragment sizes about 136750 bp generated based on RAPD-PCR analysis as shown (Table, 5), 67150 bp for subculture 1 and 69600 bp for subculture 6. The proximity matrix between the two subcultures 1 and 6 was about 96.5%.

PCR represents a major advance in DNA analysis allowing the detection and identification of a segment of DNA or RNA that occurs in extremely small quantities within a sample.[25] we used the same technique for conformed the viral infected banana plants.

Tissue culture of banana as a mean developing virus-free plants from infected stocks has developed. Three methods have been used to produce plants free from BBTV and BMV. They are meristem tip culture, thermotherapy and chemotherapy. Many workers used meristem tip culture technique to eliminate BBTV and BMV from infected banana tissue and production of virus-free plantlets The production depends on some factors, the most important factor is the meristem size. The smallest the sizes of meristem tip (1 mm), the highest percentage of virus-free plantlets obtained. But the biggest the sizes of meristem tip (4 or 5 mm), no virus-free plantlets obtained in both viruses. This result is in agreement with[4,16].

Our results are in agreement with the success of meristem tip culture depends on the nature of the virus present. Some viruses are more readily eliminated than others. The distribution of the content virus within a diseased plant varies greatly. The reason for this is the following factors, i.e., high metabolic activity, lack of vascular system and high auxin concentration[2,24]. suggested that virus eradication during meristem tip that occur in the meristem due to excision injury. Enzymes necessary for virus replication may be not available in culture meristem tips for a time period sufficient to allow viral RNA to be degraded. Welkey[34] reported that the high temperature has been widely used in the production of virus-free plants. Such treatment usually involves the growing of the infected parent, or organ of the plant in hot air in temperature controlled cabinet at 30 to 40°C for periods of 16 to 12 weeks.

Joshi and Joshi[18] obtained 25% CMV-free banana plants from infected rhizomes at 60°C (wet heat) and 75% and 100% CMV-free from treated rhizomes with 35°C and 40°C for 60 minutes, respectively. They mentioned that hot water treatment to the infected rhizomes is not the practical proposition as the treatment are injurious to the host tissue, while dry heat treatment of 40°C for one day is useful in curing the infected rhizomes effectively.

The success of the heat therapy depends on selecting the temperature and the duration of the treatment according to only with the elimination of virus but also with the survival of the plant. The results presented in this study show the survival rate of the plants is higher after *in vitro* treatment than after *in vivo* heat therapy specially in fruit species which are very sensitive to high temperature[2]. Some comparative studies as reported by Gella and Errea[11] demonstrated that the virus therapy can be considered as a good method to eliminate certain viral diseases that are difficult to eradicate by other means such as meristem propagation. As this technique has widely used for obtaining virus-free plants of many plant species, but for fruit species only a certain percentage of the plants developed from the excised meristem of infected plants are really virus-free[3]. The reason for this includes the failure to eliminate viruses in explants. Lack of differentiation during tissue culture and failure to induce rooting of plantlets *in vitro*[31]. The rates of multiplication and migration of these viruses are high so they can keep up with the shoot tip growth, consequently.

Our results illustrated that the percentages of virus-free plants were increased by either increasing the incubation period or decreasing the excised meristem size these results are in agreement with those obtained by[2].

For obtaining virus-free materials, addition of antiviral compounds and/or the growth substances are recommended in the absence of heat treatment and meristem cultures, as well as for those viruses that are difficult to eliminate by heat therapy and meristem culture especially in the commercial laboratories.

Different substances have been tested for their antiviral effects and a number of such compounds were known to inhibit certain plant viruses *in vitro*. Moreover, the multiplication of the viruses is found usually decreased when infected plants were treated with chemical analysis to the purine and pyrimidine bases of nucleic acids such as virazole, thiouracil, 8-azaguanine on the other hand increased percentage of virus-free progeny. These analogues were acted like heat therapy to increase the success of the apical and axillary meristem and tip culture techniques[6,34].

Two different compounds ribavirin and thiouracil at 10 and 20 mg/l enhanced growth differentiation of the propagating cuttings due to 60, 84.7% and 42.4, 66.6% of virus elimination as well 86, 71.3 and 75, 65% survival plantlets respectively. While 30 mg/l decreased the survival rate to 20% and increased the percent virus eliminate to 91.3, antiviral compounds are too closely linked with normal metabolic processes in plants further some of substances that inhibit viruses appear to be phytotoxic or cause mutation in the treated plants[21].

Acetyl salicylic acid (ASA) or sprain or polyacrylic acid activities play a role in the induction of BAR in plant after pathogen attack. Its inhibited virus replication and induced their near proteins (RP) in the treated plants. Which might induce resistance to the virus in plants through the synthesis of an antiviral factor[1]. It has been proposed that SAR is mediated by an endogenous

signal that is produced in the infected leaf and translocated in the phloem to other plant parts where it activate resistance mechanisms[35]; it acts by minicking an endogenous phenolic signal that friggers PR gene expression and disease resistance[23]. It stimulated the induction of peroxidase, superoxide dismutase and glycine rich wall protein. Such compound were induced by pathogens and was though to function in defense[19]. Ribavirin (virazole or 1-b-D-riboribofuranosyl 1,2,4-triazol-3-carboxamide) a base nucleoside analogues uracil, adenine, thiamine.

Generally using virazole at different concentrations led to freeing banana plants infected with BBTV and BMV. Results demonstrated that virazole and salicylic acid at concentration 10 mg/l enhanced growth differentiation of propagated meristem, while decreasing of percentage virus-free plants. It can be concluded that, using virazole and ASA at concentrations ranging between 10 to 40 mg/l (in BMV) and 10 to 30 mg/l (in BBTV) combined with the procedure of tissue culture which previously described in this work lead to great success in eradication of BBTV and BMV and increase the percentage of virus-free plantlets, El-Saghir[10] obtained the same results.

The aim of the present study was to provide polymorphic RAPD markers suitable for detection of somaclonal variations in tissue culture banana derived plants[32].

Using RAPD-PCR fingerprinting revealed correspondence between banana plantlets (subculture 6) and original plant (mother plant) of vegetative growth after transplantation on the field. RAPD analysis of two samples where closely related showed genetic variations in approximately 4% of the analyzed plants (10 regenerants). The genetic variations were not detected in subculture 6 and mother plants. Such observation suggests that the subculture 6 is exposed to selection programs of tissue culture. It could be concluded that RAPD can be successfully used to detect somaclonal variations among *in vitro* regenerated banana plants derived tissue culture. Numerous researches proved that the sensitivity of RAPD was sufficient enough to detect genetic changes in many of tissue culture derived plants; for instance, Mahmoud and Sawahl[22], Brown *et al*.[5], Taylor *et al.*[32], Rani *et al.*[28] and Saker *et al.*[30] in Garlic, sugarcane, populous, wheat and date palm, respectively.

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